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EFFECTS OF RADIOFREQUENCY ENERGY (RF) EXPOSURE ON MICRONUCLEUS FORMATION

VOLUME II

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P R O C E E D I N G S

DR. OWEN: To refresh your memory, the purpose here is for us to take your input on the type of scientific questions that have arisen from the available research that has been done on radiofrequency exposure and micronucleus formation.

In this morning's discussion, what some of us did last night and early this morning was to pick through yesterday's discussion and try and identify the major topics that we hit upon. The idea this morning is to over this draft list and, hopefully, basically complete the list by the end of this morning's discussion.

The list, as I say, is general topics of the type of question or issue that needs to be addressed and then we would like to pair with that, of course, the types of experiments that flow from that issue.

I just introduced what we are going to do and, actually, because you and Dr. MacGregor were able to spend the last few minutes fine-tuning this, I am going to ask you to help us start with our list of topics that we have worked up together.

This is the list. It will take some time to run through. The first thing, as I recall, on the list is actually the basic idea of the need to repeat studies. I am

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consciously not using the word "replicate," but using "repeat" as the term.

I think that what we got out of yesterday's discussion was that it would be worthwhile, at least, to get some number, N, repeats of the available bioeffect with, of course, certain definition of conditions. To my recollection, at least twice was what somebody said. I would like anybody to offer clarification or correction as I go along with this.

So, actually, I guess we said reproduce the findings in other labs was one way to put it.

DR. LOTZ: And in other labs meant do it again.

DR. ROTI ROTI: I would like to just make sure we have the existing repeats established. I just talked to Graham and the WTR ILS team has two repeats on all of the 10 W exposures, one repeat on the 5 W exposures. All of our data was repeated at least three times and the positive differences were repeated six times.

So, as you are planning to request repeats, you should be aware of the repeat status of the existing studies.

DR. OWEN: What I would like to ask for in return is copies of the manuscripts in confidence that we can use to discuss further and to, as you say, incorporate into that.

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DR. ROTI ROTI: At the end of the discussion, you can have our draft manuscript, but I just wanted to hang onto it until I have answered all the questions.

DR. OWEN: Okay. I understand. If I could get the same thing from you, Graham, that would be very helpful.

This list is not in any particular order. Actually, along the lines of the point that Joe brought up, I wanted to ask other people in the room if they can give us any information about other studies, other RF micronucleus studies, that are already being planned or are ongoing.

DR. FENECH: A point of clarification from the data that was shown yesterday. There is some confusion as to the extent of the micronucleus frequency that occurred in the studies that were positive. Was it an increase of 1 per 1000 cells, or was it 10 per 1000?

DR. HOOK: It is 10 per 1000, 1 per 100. We presented our data as frequencies in numbers per 100. So it was 1 per 100, or 10 per 1000. Since we actually scored 4000 cells, it is that difference that was used for the statistical analysis, the actual numerical difference.

DR. FENECH: Okay. The actual numerical difference is an increase of 10 micronuclei in 1000.

DR. HOOK: Per 1000; right. We scored 4000 cells so it was like an increase of 40 micronuclei in 4000 cells.

DR. FENECH: Your results, Joe?

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DR. ROTI ROTI: I was between--the easiest way to remember it is a third and a half a percent. So, if we were to go from 2.5 percent or, let's say, 3.0 percent, it would go up to 3.36 percent. So the difference we had was really small. Let me just give you the picture.

DR. ELDER: Graham, your numbers come from your 0.5 percent control value and your effects level was, like, 1.5 percent. That is where you get your 10 per 1000?

DR. HOOK: Yes; in that one particular experiment, that is what it was. Our average increase was, I guess we are saying, about a four-fold. The frequency in the controls varied from 0.5 to--well, actually, I think we had one case that was 0.2 percent--to something like 0.7 or something. 0.5 I think was the highest--

DR. OWEN: Was that, for the moment, sufficient clarification on your question?

DR. FENECH: Yes; it is sufficient for clarification in terms of trying to understand the biological importance of the change.

DR. OWEN: Absolutely. Actually, one point further down our existing notes that might fit well at this point too is that there was some discussion yesterday of the statistics used. I think even a comment that there was probably a lot more yet to be gained from the available data on further analysis.

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So, while I don't recall anybody explicitly recommending this yesterday, I think it might be worth considering that additional statistical analysis of the available data by the different available or already used methods might be fruitful.

The next, going down in order, we have to extend the range of the dose-response evaluation--as you know from yesterday's work, to characterize the currently available data as a dose response is, I think, as Drs. Tice and Hook described, probably reaching a little bit far. I think there was, from yesterday's discussion, some agreement that, as part of the future work, a more thorough look at the relationship between the response and the dose rate would be merited.

DR. ROTI ROTI: Is that open or are you going to make a recommendation of the dose ranges? The logical progression is 5, 10 and 15 W/kg. Getting above 15, we start getting into other questions. It makes a little bit of difference in the technology we use to irradiate.

DR. OWEN: I think that when FDA completes our recommendations, and I didn't mention this morning yet but I think I mentioned yesterday that, after we receive all this input, it is our intent to compile the input as advice that we give to CTIA to use to write requests for proposals as you are alluding to.

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I agree that we want to have more than just, say, do dose-response evaluation. I have what you just said about 5, 10, 15. I recall yesterday some discussion of scanning the range of 1 to 10.

DR. ROTI ROTI: With higher count frequencies; right?

DR. OWEN: Yes; that was mentioned yesterday. Very good. Higher counts at the lower levels or an approach that gives higher counts at lower levels.

DR. LOTZ: Joe, I want to ask; as Russ and I were talking last night, I felt like our discussion yesterday was saying that 15 may not be within the range of what we can do with sort of current technology of exposure systems, at least with any reasonable temperature control at all.

DR. ROTI ROTI: That is a concern; yes.

DR. LOTZ: So I guess I was wondering whether, from a practical standpoint, in any near-term situation, anything above 10 was very feasible.

DR. HOOK: C.K. can talk about our system better than I can, but my feeling is that we certainly should be able to go higher than 10 and control at 1.

DR. CHOU: This is all power-dependent, depending on how big a generator you have. The Kalmus can go up to 1 kW. It can even do to 100 W/kg. But whether that is necessary or not. Also, of course, the higher the SAR, your

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thermal gradient will be so big. And the cooling will be very difficult depending on the volume of your sample. That is a compromise to be made somewhere.

DR. TICE: My only feeling right now is, based on the kinds of things we have, I don't know if it would be overly useful to extend it above 10. I just think you are going to end up--

DR. OWEN: I'm sorry; to--

DR. TICE: I don't know if I would extend it above 10 just because of all the other kinds of constraints that are going to start driving it. We see a response at 10 and 5 in the sense that we feel that, even though we didn't reproduce the 5 within the same technology, 5 was positive in two technologies. So that is some kind of replication, given that the 10 response is replicated across.

So we already think that there is a dose-response relationship within that range from 1 to 10. Going above 10, you might get higher numbers, but I don't know if it would provide any more information.

DR. ROTI ROTI: I just talked to Eduardo who has recently proposed optimizing the RTL which, if that were to be successful, in about a two-year span of time, we should be able to do 10 W/kg in an RTL and then you would have a bit better temperature control, I think, because the optimization would have failed if we didn't have that

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temperature control and the advantage to going above 10 is that, right now, you are going between, as we said, 3 and 10 micronuclei in a 1000 as your delta increase.

If you could get up to 15, you could theoretically go--you would be expecting on the order of 15 to 20. So it would give you a bit better statistic. It is something to consider, I think, in term of planning the research. If you would want to add some details about the optimization and the requirements, I think Eduardo could probably answer that.

DR. OWEN: I think we will discuss that further. I think we have a separate item that relates to that a little more directly, perhaps.

DR. LAGROYE: Russ, to answer your first question about what is done in other labs about micronuclei, we have a study which should be out in the fall, and we will be repeating Dr. Verschaeve's study with the human lymphocytes with the 900 GSM and also the 1800 GSM.

But we plan to use pretty low SAR, 0.4 and to go up to 2.0 W/kg. Also, the exposure time was supposed to be up to 2 hours, so maybe we could extend--

DR. VERSCHAEVE: Is it micronuclei?

DR. LAGROYE: Yes.

DR. VERSCHAEVE: Because our study was cystochromatid exchange.

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DR. LAGROYE: Yes; but it is an extension. Cystochromatid exchange is also included, but we plan to do the micronuclei also.

DR. VERSCHAEVE: Only because you say a repeat study.

DR. LAGROYE: It is an extension; I'm sorry.

DR. VERSCHAEVE: Maybe I can add also that we are involved in a study that is sponsored by the European communities. This is a study that is a long-term cancer study in rats but we will do a micronucleus test on the blood let's say about every six months because they are exposed during two years.

We will do a micronucleus testing combination with FISH for aneuploidy detection. At the end of the study, when the animals will be killed, we will also do--no; we do comet assay also on the blood but, at the end, we will also extend the comet assay on brain cells and other tissues.

I think that, in the same program, the team of Maria Scharfi in Italy is doing in vitro studies in which he also will do a micronucleus test. I am not sure anymore, but I think micronucleus test is included also. That is 900 MHz and I think also 1800.

DR. OWEN: You said that Scharfi's work is in vitro?

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DR. VERSCHAEVE: This is in vitro; yes. Ours is in vivo, but she is doing in vitro.

DR. OWEN: On each of these three studies, I am curious as to how firmly established the protocols are. In other words, would these studies be informed by what comes out of this meeting or are they all pretty set?

DR. VERSCHAEVE: Normally, they are set.

DR. OWEN: Okay.

DR. FENECH: I would like to make on the dose range, on the range of dose-response evaluation. One aspect of concern that I have is the heating aspect of the exposure depending on the SAR. So, in other words, if the current in vitro systems, setups we have, are adapting to the heating more rapidly than the in vivo situation, then we don't have a system that can properly evaluate the effect that might occur in vivo.

So it seems to me that there is a bit of a flaw in the experiments that have been done so far in that they have not included the test without the compensatory cooling. So I personally would like to see that in the future experiments you also do the test without the compensatory cooling to the point that is feasible.

DR. MOROS: Wouldn't it be better without the cooling, you are saying?

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DR. ROTI ROTI: No. I think a better thing, rather than without the cooling but rather with controlled temperature rise so that we have a reproducible temperature rise which we--the way this experiment would have to be done is that we would have to pick a temperature rise that would be logical with that SAR.

We would have to do the hyperthermia experiment at that temperature rise. We would have to do the RF experiment without the temperature rise and then we would combine the two. It is a really three-armed study. But it is a very reasonable thing to do. But you would have to put that in your request for the proposal, I would think.

DR. CHOU: Joe, I think in addition to the heating, a control study is important. Also the direction is cooling because when you blow the air in there, there is at 0.7 degrees maximum differential between the sham and the 10 W/kg, at least in the system Ray and Graham used.

So lower and higher, whatever, in that range, you have to be tested with a positive control, temperature control.

DR. ROTI ROTI: So this is a "when is the temperature not the temperature" kind of study.

DR. OWEN: I would like to follow that discussion further, but I want to ask first if Dr. Swicord's comment

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has to do with what we were talking about before or whether it is about the non-RF heating.

MR. SWICORD: No; it is going back to the question of who is doing what in terms of micronuclei studies.

DR. OWEN: Could we take that comment and then we will go back to the--

MR. SWICORD: Just an addition. You asked whether or not there could be some changes in the protocol. The work that Isabelle was talking about, there are two laboratories that are involved and the contracts haven't been really finalized on those at this point. They are close to being finalized so it is possible to rapidly, perhaps, do something about those protocols.

DR. OWEN: Thank you. Now, let's please resume discussion. Actually, what we had on the list, and I am not sure I see it right here, but there were two main areas, I thought, of dealing with the questions related to heating. I think we are right now talking about the idea of conducting experiments with different starting temperatures. I think one person characterized that as an ambient temperature offset.

What I have heard just so far this morning, again, is the need to do--the suggestion that there is a need for experiments without cooling, experiments with cooling all

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samples down to a lower start temperature so to speak so that the SAR rise is offset.

DR. ROTI ROTI: I think it is important to adapt the cells to a lower temperature before you do that experiment. It is not the same thing as to just cool them down and then do the experiment.

These kinds of studies have been in the heat-shock field for a number of years and the key has always been a cell adapted to a lower temperature is then more sensitive to a temperature increase. I think as a way of making sure the system is reproducible requests that cells be adapted to the lower temperature.

DR. TICE: How do you do that?

DR. ROTI ROTI: I don't know how you do that. But why would you want to cool the lymphocytes before you start anyway. They are supposed to be at 37.

DR. HOOK: You are not trying to cool them. You are just running the exposure at a lower equilibrium temperature so that the idea, as I understand it, is that this heating that we are going to produce from the RF will only bring them up to a temperature that would not induce micronuclei. That is the experiment.

This is introducing, I think, a lot of extra--

DR. ROTI ROTI: I could agree with that, but I am just saying if you are going to reduce the starting

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temperature at exposure, it really ought to be done with adapted cells.

DR. OWEN: Perhaps we should talk less in the abstract and say what temperatures would be intended for, say, the offset experiment. In other words, the target temperature thus far has been 37. What temperatures might be targeted?

DR. MOROS: From the physics point of view, the starting temperature is not really the important thing physically--it may be biologically--is the steady-state temperature that the sample is at for either 3 hours or 24 hours.

The target temperature has always been 37 degrees. Everybody agrees with that. I think that, for most of the exposures, SARs, that we are talking about, that is feasible. It may be that when we are getting closer to 10 W/kg, there may be a difference between the shams that don't have power deposition and the ones that do have power deposition.

In that case, I think the suggestion of C.K.'s is very good. If you find that there is a consistent difference that you cannot compensate for because you are just heating too much--in other words, you cannot get the sham at the same temperature--then you do the hyperthermia experiments to sort of have another control.

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But I don't understand what you mean starting at a cooler temperature. I think what your meaning is to have your environment at a cooler temperature. That is what you are meaning.

DR. HOOK: It is what you said, the equilibrium temperature. First, we don't have all of the information we need to say for sure what it should be because we don't have the good heating without RF MN information. From what have, if you looked at the literature, and most people are talking about 39 to 40 degrees C before you start seeing an effect, that is looking at about a three-degree temperature increase.

So, based on that, if we say, since we saw micronuclei and you need a three-degree temperature increase, you would drop it down by three degrees. That seems like a lot but the other side is what C.K. has been saying about system where, in fact, based on what extra cooling we have to apply to a 10 W situation, it is only 0.7 degrees C.

DR. CHOU: That is because, based on our 1979 study, it showed three bars. We saw we had good cooling and we saw the temperature was constant. So we saw a big change, a big effect. We thought it was a nonthermal effect until we figured out that there is a big thermal gradient, very, very difficult to measure because it is such a sharp

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gradient, at the bottom where the cells are--you cannot measure with a point temperature sensor. You measure and you are still getting the average at the bottom. But this is across a very thin layer at the bottom.

Until we pump the cell up, we expose that and it is another effect. So I am trying to say here is using the same approach here. We know there is a difference between the two systems when you use cooling. You blow air into there is different.

Maybe that small difference can show. I don't know. That is why we need the positive control experiment. Maybe you blow this at a different temperature without any RF and see if you see any effect up to 24 hours. I don't think any biological data exists nowadays to show at different temperatures do you see a difference for the different samples at different temperatures.

That is something we definitely need.

DR. OWEN: Yes; I think that is one of the major points that we have had throughout the discussion is that there seems to be a need for a series of experiments that would define the response of the micronucleus assay as it is conducted here, in vitro, to non-RF heating and I guess to not only say heating there but actually say it is the response to temperature to allow for collecting data below 37 as well as above 37.

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MR. BASSEN: Is there a possibility to stir by rotating or tilting every half hour the dishes to get some sort of temperature gradient at the boundary, at the base of the plastic, removed?

DR. CHOU: That is the problem with this system because this is a test tube. If these cells start floating up, your cells are going to be exposed to a very wide range of SAR. That is why we keep that all spinning down to the bottom.

MR. BASSEN: Right. I don't mean completely inverted but just a little bit of movement to get the temperature, the heat redistribution.

DR. ROTI ROTI: I think we need to go back. We can agonize over the temperature artifacts in one system, but the chances of the temperature artifacts being the same in the WTR system and in our system with their very different geometries are very small.

I think the solution is to do the studies at both places, or with different exposure systems because the chances of the same temperature artifacts being present--we can spend forever brainstorming how to get the temperature artifacts out of those test tubes.

DR. OWEN: I think, Joe, that was the first point that I made.

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DR. ROTI ROTI: That is what I am saying. Let's go back and strengthen that first point.

DR. OWEN: I would rather go through the points we have in a certain level of detail before we get into finer detail on any one point and don't have enough time left to deal with the remaining points.

DR. ROTI ROTI: Okay; that's fine.

DR. WILLIAMS: It seems they are related. The dose-response experiments should have two purposes. One is to drive things, perhaps, to a higher SAR so that--with the hope of better validating that there is an effect and, if possible, to separate thermal and thermal effects at that level.

But the other purpose is to say something about shape. Is this a threshold phenomenon? Is this a stochastic phenomenon? Ray, in your work, I don't remember, the 5 and 10; was the 10 higher than the 5 in the system? Did you have the indications?

DR. TICE: In one experiment, the 5 was about halfway between the control and the 10. In the other experiment, the 5 was the same as the 10. In both cases, we did 1, there was about a--what is called maybe a 10 percent increase above the control in two experiments but they are not statistically significant and probably not even relevant.

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DR. WILLIAMS: But no one has ever seen anything at 1.

DR. TICE: In those two; no. In those two experiments based on the number of cells scored; no.

DR. WILLIAMS: It seems to me that one effort has to be to go to a lower SAR with a better statistical handle on that.

DR. MOROS: One more comment on this. I hate to come back to the same subject, but it is important. The setup done in the Crawford cell by Hook and Tice with the test tubes will sustain larger thermal gradients that are set up in the RTL. I am not saying that they cannot control their temperatures of the sample to what they want. What I am saying is that, since the test tubes are in air, there is a larger temperature gradient between the inside of the test tube, within the test tube, with reference to the air flowing inside the Crawford cell.

We don't know whether there is an effect of temperature gradient. I don't know if cells exposed to a temperature gradient would have an effect. That is something that needs to be said as background information.

DR. TICE: But the thing to also reflect on is that Joe already has results at 5 W/kg using the system that you have which, as Graham said, suggests that because both systems give about the same magnitude of response under

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those conditions with having very different spacial orientations and heating gradients, that if it is heating, it is something more than just a gradient. It is not quite the same. Something is independent in that process.

DR. OWEN: I think inherent in the idea of repeating the experiments that are available is that more than one exposure system would be used--

DR. TICE: Absolutely--

DR. OWEN: To see if the same nominal exposure conditions give the same or very comparable results, taking into consideration, of course, the possibility of the impact of the biological sample that is being used.

DR. TICE: Russell, the other thing I would like to mention is that I think we should look at some of the experiments we are talking about is not as if they are in a vacuum and that is the only experiment because different experiments can give you different pieces of information that, together, make some kind of what is called--the typical thing we say is weight of evidence.

I would actually like to see the cells run at, I don't know, 34 degrees and see whether or not we see the same increase at 34 steady-state temperature as we do when we run them at 37. I don't know that a negative at 34 means something but a positive at 34 would mean something to me in terms of the kinds of response we are talking about.

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But it is very easy to argue about one experiment because no single experiment will answer everything that we are trying to ask.

DR. OWEN: Right. One of the other important points about the emphasis on getting repeated experiments is that a number of excellent hypotheses are being generated by comparing the available data from the two systems but to further flesh out these hypotheses or confirm them requires, I think, more data.

DR. TICE: Absolutely.

DR. ROTI ROTI: We did 3 W and we found no evidence of an increase at 24 hours in either of the samples at 3 W/kg. You can also probably adapt the irradiation conditions to run cells in test tubes in these RTS because we ran some binding reactions that were run in Epindorf centrifuge tubes in the RTLs. There is a way to modify--they have to be short tubes. But it might be possible to run cells in tube and in monolayer in the RTLs.

DR. OWEN: I think that is a perfect segue into one of the major points that we have from yesterday's discussion that we can continue to flesh out today and that is what questions there are regarding exposure systems as they exist and as they might be used.

A blanket way of saying this is that we need to use a good exposure system, a good exposure condition. I

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would like to continue this discussion of the capabilities of the exposure systems now to get down some of the points that might be criteria for evaluating whether an exposure system is sufficient for the experiments that would be requested.

DR. WILLIAMS: If you were to compare your two systems by looking at the frequency distribution of SAR with the voxel temperature, would they be greatly different? I remember the one where you had SAR across the bottom and voxel frequency. How different would they be between the two systems, or would they?

DR. MOROS: Between the--

DR. WILLIAMS: The two different exposure systems.

DR. MOROS: Oh; I would have to look at the data closely. I haven't.

DR. WILLIAMS: The other thing I suppose I am asking is that, in the two systems, you have certain cells that are at a certain voxel and experiencing certain SAR. They would be comparable? In other words, the only difference is the rate of heat change, the ability to cool, the ability to get--you get a different distribution of SARs within your experiment samples. I guess I am going to ask, if we compare those, is there a basic difference between the environment an individual cell sees if it exposed at an SAR of 5 in on system and an SAR of 5 in the other.

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DR. MOROS: My first guess is that there will be a difference in terms of average SAR and standard deviation within, in my case, the cell layer. In my case, it is the cell layer. In their case, it is a pellet. We mapped the temperature in our cell layer and it was uniform. The SAR, we did measure it and we also have calculations but I would have to sit down and compare the histograms to say something about it.

They will be different, I think. I think they will be different from flask to flask and they will be different from RTL to RTL. But I think they will be even more different when their cells see and what our cells will see.

DR. WILLIAMS: It just seems that is going to be the basic--I guess what I am asking is, comparing the two systems, what are the parameters you use to compare biological responses? One of them would be the distribution of SARs among the different cells. What I am asking is is that enough if we say that your system, on the average, at a certain average SAR has an wider or narrower distribution and, therefore, there are fewer cells at the higher range of the SAR.

That is what I am asking is how do we compare the two systems.

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DR. ROTI ROTI: Eduardo, we have the voxel distributions or the SAR distributions on percent of cells from the WTR data. We would see if you have that for our monolayer and then we have to compare those distribution plots because the issue is the fraction of the cells at the extremes.

I think Eduardo could do that. We don't need to repeat. We just need to get that from their data they already have. It is just a way of reconfiguring that data.

DR. LOTZ: One of the questions that was discussed some yesterday seems like it fits in here and that was whether there is a need to try and some more extensive--I will call it thermometry to measure temperatures to try and put some empirical validation to those calculated SAR findings.

I see Graham shaking his head no. You are one that I remember talking about. You and C.K. were talking about measuring and how close you could measure to the bottom of the tube, and things like that.

DR. CHOU: Myself, I have been doing this work a long time ago on the nervous tissue. Of course, they are at the organ level and not the individual cell level. The most important thing at that time we found is the only difference is the temperature. You say assay up going up to 20 W/kg,

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that king of a peak-power exposure, or even CW, up to 1500 W/kg.

You can boil the tissue, as long as you keep the temperature constant. Even down to 0.2 degree temperature difference, I was able to see the difference on the nervous response. Here, on the cell level, it is probably very sensitive. I don't know. So we have to be very, very careful on the temperature situation.

DR. HOOK: I think the question is would be expected to accurately measure temperature difference that would correlate with the calculated SAR values we are seeing in our exposure systems. My thought was that we just can't do that. We don't have that accuracy in our thermometry.

DR. OWEN: There was some discussion yesterday of doing measurements on phantoms to help address that question.

DR. TICE: But the thing that came out of that was, at high SARs, when we are talking about high SARs, you could probably do that. But at the SARs we are talking about, by the time you open the phantom, what C.K. said was that the temperature resolution would actually disappear that quickly.

DR. OWEN: But, I think, as was discussed also by C.K., that is a standard way of looking at things when you run into that problem is to look at higher SARs and see, and

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to do the modeling to see how the modeling compares to those phantom measurements at a higher SAR.

DR. CHOU: In the past, we used a phantom. We always try to detect the SAR pattern by using the temperature method, to use the Luxtron non-perturbing temperature sensors, or expose the biological object with a high-intensity power and a short time to minimize diffusion, and we try to capture the SAR.

But now we have the SAR pattern by using the FDTD calculation. That is the worst case of temperature pattern, right there. After that, then you just have diffusion, whatever, the size of the object and the perfusion, whatever, convection. That will change the final temperature rise in the sample.

DR. MOROS: I think that the point in the middle of the board there about modeling, one thing that can be done is to model their--since they already have the SAR distribution within the test tube and, presumably, there is no SAR outside the test tube or in the physical material in the test tube, you can construct a three-dimensional thermal model that will take into account the boundary conditions and the ambient temperature inside the Crawford cell and the thermal properties of the material, and then at least come up with a very--I would say a fairly robust temperature

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distribution inside and at least that will tell you the area where you can expect 37 degrees.

I think that is not unreasonable to do the temperature measurements that I suggested yesterday of just mapping the temperature using the Luxtron by pulling up the sensor at steady state and just mapping what the temperature is maybe in the center line.

That sort of center-line measurement can then be compared to the modeling to verify modeling. I think that is something that is feasible to do.

DR. CHOU: Engineeringwise, that is the simple approach, to do the FDTD calculation and couple it with the heat equation to get a predicted temperature, final temperature distribution. Then verify with your temperature mapping to verify, see how the two compare.

The other one, in terms of biological, I think it is simple to do the control study, run different sets at different temperatures with RF, see how they respond. Are there differences from one temperature to the other within that temperature range.

DR. TICE: One of the things to reflect on, and maybe it is because our backgrounds are biology, not physics, when we were assessing--we had two probes in the tubes that were doing the temperature. Based on the

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distribution of SARs for the whole tube, one thermometer, one measuring device, was where the cells were.

But the other one was where the maximum SAR was in that tube. The temperature difference between those two spots, again, based on expectations, was half a degree for all experiments that were done except with PCS which was 1 degree.

So right there, that tells us, at least to me, what the maximum difference in temperature is that we are going to locate if we go through and do this, measuring physicalness throughout the whole tube. We already know what the temperature difference is, but that temperature difference, the ability to measure at a smaller volume, because a voxel is smaller than probably the probe can even measure, isn't going to give us a bigger temperature difference than that, and that is already less than anything you would expect to cause hyperthermia because, again, they are running at 37 degrees. Hyperthermia is at 39 or 40.

I don't know if we can physically measure it with what you are talking about. It seems to me that what Joe was talking about yesterday using a biological response indicator of hyperthermia would actually be more informative although the problem there is you could say what fraction of the cells show a response by expressing whatever the signal was, but, at least in our system, you wouldn't know where

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those cells were located because, by the time you take them out and do the measurements, they are no longer in the same spacial orientation they were in the pellet.

But at least you would know what fraction of the cells at any one SAR show, as long as you know what the threshold temperature is that causes the response, you would be able to tell where those cells are.

Is the magnitude of the response within a cell proportional to the temperature?

DR. ROTI ROTI: It has all got to be worked out.

DR. TICE: So I am just saying biologically it might be a better way. You would always go with both prongs because both capabilities exist. But it just seems to me that you tie those two things together and you would probably get your information.

The question is, looking at it from even another side, what does that information give you at the end result? What are you going to do with the information--

DR. OWEN: You have something to put together with the information from the non-RF heating experiments, presumably.

DR. TICE: But if you go through and mechanistically determine the origin of the micronucleus so that you know that, when you measure the micronuclei-induced by raising the ambient temperature and you look at the

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frequency of the micronuclei-induced as you raise the ambient temperature so you know what fraction of the cells are responding, theoretically, you get a higher fraction of cells because it would be more at a uniform temperature but you would be looking at the micronuclei to see that, mechanistically, the origin is the same, and then try and titrate back to what is going on from that particular fashion.

DR. ELDER: Let me make some comments as to why a temperature difference of a half a degree in that cell system may be highly significant. If I understand this process, you have got the blood cells in that bottom 1-ml--

DR. TICE: Bottom one-third.

DR. ELDER: Over a period of 24 hours, this test tube is sitting essentially stationary, no agitation. Over a period of 24-hours, these cells are going to continue to sediment, clump towards the bottom. In fact, I would be curious to know, from a 3-hours exposure to the 24-hours exposure, do you see any visible difference in the aggregation of the cells in the bottom of the tube.

But that is not what I am getting to. This process that gives you micronuclei requires that the cells go through a cell division in that period of time.

DR. TICE: No.

DR. ELDER: No?

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DR. TICE: No. The cell division is post-exposure.

DR. ELDER: Post-exposure.

DR. TICE: The cells are sitting there quiescent. They are not dividing. It is lymphocytes and leukocytes. It is leukocytes from your blood sitting at the bottom one-third ml.

DR. ELDER: I think what I am thinking about would still hold. During that 24 hours, these cells are clumping. I would think that ones in the interior of the clump would be becoming oxygen starved as well as metabolically starved.

DR. TICE: But you have a control. The cells in the controls are doing the same thing.

DR. ELDER: But, in your exposed sample, you have got a half a degree temperature difference.

DR. TICE: Not there.

DR. ELDER: In various parts of that small volume.

DR. TICE: No. The half a degree temperature difference is between the bottom of the tube and the top of the tube. The temperature difference at the bottom of the tube is 0.2 or 0.3 degree over that period of time.

DR. ELDER: That is getting close to half a degree, 0.2, 0.3 degrees. I am suggesting even that small temperature difference over a 24-hour period to cells that are being oxygen starved and maybe metabolically starved

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would contribute towards increasing the number of nuclei in that post-exposure, after exposure when the cells go through the cell division.

DR. TICE: And so, by lowering the temperature at which the cells are exposed would obviate that problem.

DR. MOROS: No.

DR. ELDER: No; I don't think so. I am just trying to suggest a possible explanation for why you might be seeing increased numbers of micronuclei in this particular setup.

DR. FENECH: The settling is not a problem. From in vivo and in vitro experiments with ionizing irradiation, if you expose the cells in vivo or in vitro you get the same dose response more or less.

DR. TICE: It also doesn't explain Joe's data which is a monolayer.

DR. FENECH: This is exposing before you culture the cells in a test tube.

MR. BASSEN: I would like to make a point that the statement was made that the half a degree temperature rise is because you measured at the location of maximum SAR versus the bottom. Well, temperature is not equal to SAR. the highest temperature is likely to be at the top just because of convection.

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Temperature is not equal to SAR in the steady state unless you have some--the SAR is only the measurement of temperature over a few seconds, the rate of temperature rise. So measuring at the hot spot of SAR will not give you the highest temperature in the test tube.

DR. CHOU: I think the temperature at other areas, other than the cell, doesn't make--is irrelevant because cells are not there. So I am really talking about right at the boundary, the bottom of the tube where the cells are most are near that area. It is very difficult to measure exactly where the temperature of the cells--because it is such a small, thin layer and all the cells pack in there.

All I am saying because there is a difference in the cooling temperature up to a 0.7 degree difference, you keep constantly going over that area for 24 hours, can that small difference in temperature--where most of the cells are at the bottom, can make a difference? That is just the question.

MR. BASSEN: I mention that you if you stirred, that is obviously not a good idea biologically. But if you could bubble air very slightly to move those cells once an hour, say, just to circulate them so that they are not sitting in a hot spot or a temperature gradient, as you mentioned, right at the bottom of the test tube--

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DR. MOROS: Before you go into any modifications of what you are already doing, the cheapest way, again, is to model this numerically. We have the SAR already. We can rerun the SAR at finer resolutions if need be. We can have a thermal model and we can see--I think that is a very good point of C.K. We can see what is to be expected when cells are sitting at the bottom of the test tube.

They are closer to the outside temperature than to the hot spot maybe a couple of centimeters up within the test tube. They are closer to the ambient temperature which actually may be cooler at 37. The reason you want to do this is because, regardless of the biological regardless, then nobody can come back and tell you, "Oh; you did this wrong. You didn't think about this. Your dosimetry is not complete. Your temperature distributions are in question."

If you do the dosimetry and the thermal dosimetry well at the beginning, whatever the biological response, you cannot go back to this, the beginning, and then criticize it. Everybody agrees that it is a good work.

DR. CHOU: This biological and engineering, this totally can be done parallel. What you are saying is it definitely can be done. Even in the early '70's; actually, Emory, at the University of Washington, he did a temperature around the eye ball with the air cooling at the temperature

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and the blood flow in the eye ball. He predicted exactly what we saw in the rabbit eye; you cause cataracts.

The location and all that part is very, very accurate. I think we can do the same thing here. You do the engineering calculation measurement and the biologists to do temperature, run at different temperatures, and we can come up with a very good answer to that.

DR. OWEN: I would like to go to the point--and this was touched on indirectly in some of the discussion that just went on. I think Ray mentioned mechanisms of micronucleus and I think that that was one of the points that we might be seeking clarification on.

So could I get some elaboration of that? There was some discussion of kinetic or staining, of using FISH, and so on.

DR. TICE: What is key, in part, to interpretation of dose-response data is the mechanism of formation. Basically, what that would do is--and also you have to be aware that the mechanism of formation can shift as a function of the dose because that has been shown for some chemicals.

So you wouldn't want to just pick on dose and do mechanistic studies on that. But you would want to go through--and, generally, what would be done is to score 100 micronuclei at each dose including the control. So,

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obviously, it takes longer when you are talking about the control cells than the ones exposed to 10 W/kg, again assuming that the response is replicated.

You would go through and you would measure how many micronuclei have a kinetochore versus how many don't. It is pretty straightforward. If you are really extending the data, you might want to see whether or not you get the same proportions as a function of doing different wavelengths to make sure that there is a consistent pattern there.

But once you do it once, you don't need to keep on doing it. It is sort of like a one-shot experiment.

DR. OWEN: But it hasn't been done yet; right?

DR. TICE: But it hasn't been done yet. The only data that you have that reflects on that is that V-79 study done by the scientists by the scientists in, was it Zagreb, where they looked at the size of micronuclei and found a distribution that was consistent with chromosome aberrations, with structural aberrations.

The problem is that, in that experiment, it really does look like they had lots of hyperthermia, and they also saw increases in chromosome aberrations in the cells under the same conditions that they got the micronuclei. So that is internally consistent, but that is the only data that is running around.

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DR. OWEN: And your data were all negative for chromosome aberration?

DR. TICE: There are two things. The chromosome aberration data that we did was in cycling lymphocytes exposed for the same length of time, but they were PHA-stimulated cells where exposure started at 48 hours after stimulation.

We have not done the kind of experiment you are talking about where you take the same exposure system of cells being quiescent, stimulate them to divide and then look for aberrations. The problem is that, based on the frequency of micronuclei that we are seeing, it might be a little tough to even detect an increase in aberrations, even if one were there, because of the general differences in power between those two assays.

It doesn't mean it couldn't be looked at, but I am just not so sure that a negative answer would mean that there weren't aberrations, but we just didn't have enough power.

DR. OWEN: What about the converse experiment. I think there was a good bit of discussion yesterday, and I know this is a separate topic, somewhat, of when the exposure is conducted with respect to the stage or step of the micronucleus assay.

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DR. TICE: That referred more to what Joe's experiment design was because, in Joe's particular studies, the cycling cell situation was where he added cytochalasin after the cycling cell exposure. The statement that Jim MacGregor said was you don't know if it is negative because of the fact that it is cycling cells that are exposed or the fact that the experimental design was such that it precluded a good possibility of picking them up if they occurred, because you are looking at cells that were multiple generations down past the exposure.

So, in that situation, that would just be to do a cycling cell exposure and looking for micronuclei which could be done in both his 10T1/2 cells or in lymphocytes under the conditions where we didn't see an increase aberrations.

But you could look for micronuclei under that same circumstance. It is just that I think you want the cytochalasin B to be there during the exposure to make sure that any cell that cycles through the exposure you would end up with an increased micronuclei present.

DR. ALLEN: If you did pick up kinetochore-positive micronuclei, do you think it would be indicated to go on and do FISH as well in the main nuclei to look for non-disjunction that might be associated, also, with the chromosome loss?

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DR. TICE: If you had a micronucleus with a kinetochore--if it was a kinetochore-positive micronucleus, by de facto, you would have to have a an aneuploid cell.

DR. ALLEN: But I mean a different mechanism of aneuploidy that you could pick up in the same cells if you applied FISH probes to look for aneuploidy in the main body nucleus. You would have the cells there. It is just an additional thing that you could do to look for an ancillary mechanism involved.

DR. TICE: Jim, I don't understand what the ancillary mechanism would be.

DR. ALLEN: A non-disjunction which is not giving rise to the micronucleus. The kinetochore-positive would be a pure chromosome loss. You would get that information but there are times when that would be associated with spindle effects that would also involve non-disjunction.

So if you used the in situ hybridization methods in the main nucleus, then you could also pick up that additional information that you are having non-disjunction mechanism as well as a chromosome loss, a distinctly different mechanism.

DR. TICE: Hold on. If you have a micronucleus with a kinetochore, then that means that contains an intact chromosome. Otherwise, it would have a kinetochore.

DR. ALLEN: Right.

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DR. TICE: That means that the corresponding nucleus that is in that cell that has that micronucleus would, by definition, have fewer chromosomes.

DR. WILLIAMS: No; what he is saying is that there may be other chromosomes--

DR. ALLEN: Exactly.

DR. WILLIAMS: You may leave a maldistribution between the two daughter nuclei that do not produce the micronuclei.

DR. TICE: Right, but the thing in that particular case, though, is that--I guess what we are talking about is mechanism. It is easy to do. I am just still trying to figure out the data that would generate from that. It would give us more power for looking at aneuploidy because we would be looking at the potential for multiple events.

In fact, by looking at the binucleate cell, you could see the distribution of chromosomes between the two nuclei. Coupled with what is over in the micronuclei, you could come up with a count, as far as that goes. And that could be done. That is not a problem. It is theoretically possible. It is practically possible.

DR. ALLEN: All I am saying is that if you pick that up, the kinetochore-positive micronuclei, sometimes that is involved with lesions that are also associated with nondisjunction. You have the cells there. If you see these

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kinetochore-positive micronuclei, you would have an opportunity to then see whether it is the kind of lesion that is also involved with non-disjunction.

DR. FENECH: There is another reason why you might want to use the FISH approach. Let's say one of the effects of this exposure is actually knocking kinetochores off chromosomes. It wouldn't show up with your kinetochore assay, but it would show up with a non-disjunction FISH assay.

DR. TICE: I was trying to decide whether or not we were cooking kinetochores.

DR. FENECH: In fact, microwaves are used, actually, to alter epitopes in immunohistochemistry.

In addition, you have got the situation that, at least from current evidence with chemicals, that the non-disjunction test, within the cytokinesis block assay is more sensitive to picking up aneuploidy events than the chromosome loss. So you then minimize the chance that you get a false negative, maybe just look at chromosome loss using the kinetochore signals in the micronuclei.

So those are at least two reasons why you would want to do the FISH as well.

DR. ALLEN: In fact, even in the micronucleus, itself, looking for the centromere probe, it might be worthwhile to use both kinds of probe, either a major or

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minor satellite probe with a FISH and, in addition, a kinetochore probe and split your samples because, with this kind of an insult with heat, you could very well imagine the possibility that you are going to get a false negative--

DR. TICE: If it was negative, then that would be a concern. If it was positive, it wouldn't be a concern. So, if you did the kinetochore first and got positive for kinetochore, you wouldn't have to do centromere up front.

DR. FENECH: Yes.

DR. TICE: The other thing is you would also want to do this on cells that were cycling through the exposure.

DR. FENECH: Oh, yes; absolutely.

DR. TICE: At the same time you do it in the circumstances where we have quiescent cells.

DR. FENECH: You would do it under any constants that you would be testing.

In addition, I would also like to see, in the tests, because I think it helps the interpretation the measurement of the nuclear plasmic bridges that you have in the binucleated cells. That would provide more evidence for chromosome breakage if the micronuclei are negative, and an estimate on the same slide of the necrotic and apoptotic cells.

However, that can only be done if you really did not use hypotonic treatment. In other words, if the assay

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was done with isolated lymphocytes, you could also measure the necrotic and apoptotic cells simultaneously. That would tell you, also, whether alteration of apoptotic rate may be the reason why the micronuclei are going up or down.

In other words, if you alter the propensity of the cell that is damaged to undergo apoptosis, then it will now show up as a micronucleated cell.

DR. TICE: We actually did that with the comet assay, rather than your approach. But it is basically the same thing is to look for--

DR. FENECH: It is basically the same thing. The advantage, if you do it like this, is that you are actually getting the measure within the same population. And you can model from that information. In other words, you are scoring all the events, really, that have happened.

DR. TICE: Can you do that with whole blood, or are you limited to doing that approach with isolated lymphocytes?

DR. FENECH: With whole blood, you will use the necrotic cells, and necrotic cells occur frequently. We are talking about 10 percent of your cells potentially being necrotic. It seems to be a side effect of the cells going into proliferation.

For example, if you do the assay with hydrogen peroxide, you will find that the main event that is induced

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is necrosis, even though the micronucleus index has gone up. So all these things correlate with each other, the necrosis, the apoptosis. Apoptosis correlates negatively with the micronucleus index. The micronucleus index correlates positively with necrosis.

So these are all events that are happening simultaneously and may be relevant to the interpretation of what is the actual mechanism.

DR. TICE: Do you normally see a change in proliferation kinetics under conditions where you get necrosis or apoptosis?

DR. FENECH: With necrosis, you can see a correlation with the proliferation rate.

DR. TICE: I am just thinking of the circumstances we were looking at. We actually saw no changes except for one in proliferation, because the exposures, again, are relatively--

DR. FENECH: Sure.

DR. TICE: Do you think that the aneuploidy assessment would be limited to normal cells as opposed to transformed cells by FISH?

DR. FENECH: No; I think it should be done on any cell type.

DR. TICE: The transformed cells are, by definition, already aneuploid.

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DR. FENECH: They would have a higher propensity to become--to show a wider range of aneuploidy.

DR. TICE: It is like CHO cells are 80 percent aneuploid.

DR. FENECH: Okay.

DR. ALLEN: Again, that is controlled for. You can measure the changes in your control so, at least with micronuclei, it is a bit more difficult with FISH in individual chromosomes because of the instability. But I think a micronuclei endpoint would still be valid--

DR. TICE: No; that would be.

DR. FENECH: Yes.

DR. ROTI ROTI: I have a question I have been trying to get in here. On the request for apoptosis and necrosis measurements, it seems to me that, for our studies, the most feasible way to do that is to take part of the sample. We don't need that many cells to make slides with, but we can run flow cytometry for either tunnel assays or Annexen 5 assays to get the apoptotic fraction.

We have been doing that with Molt 4 cells and we find no effect of exposure. But we haven't done the apoptosis in the C3H 10T1/2 cells yet, so we could do that. The other thing is that we have also looked at proliferation and cell-cycle progression of these cells, not under the same samples that we exposed but under the same conditions,

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and there is no perturbation of cell-cycle progression or cell proliferation.

DR. FENECH: Right.

DR. ROTI ROTI: But the important question is that it is feasible, then, to just split the sample and do flow cytometry on part of it for apoptosis.

DR. FENECH: You could do it that way as well. As long as you are verifying that there has not been a marked change in one or the other, then you can go ahead--

DR. ROTI ROTI: But we haven't selected a subpopulation for either.

DR. WILLIAMS: We do not have a rigorous analysis of the literature, do we, on the heat induction of chromosome aberrations? There is bound to be in the hyperthermia work.

DR. ROTI ROTI: Bill Dewey did that years ago. They mostly found some very stuff of chromosome aberrations in heat. One of the tricks is to make sure all the cells--that your heating conditions are done in such a way that all of the cells get to mitosis. In fact, the original studies didn't do that. So I don't know that there has been a repeated study.

I could probably ask Joel Bedford--he probably would know this--to see, under conditions in which all of the cells actually get to mitosis because a lot of cells,

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after heat, die in late g2 before they get to mitosis--so, depending on what cell cycle they are in when they are heated. So that is a tricky thing.

DR. ALLEN: Another point about that, too, is I think there is literature that if you heat and get your stress response up, and then heat again, you have got a tremendous amount of protection from your second insult.

DR. ROTI ROTI: Absolutely.

DR. ALLEN: What is that phenomenon called?

DR. ROTI ROTI: It is called thermal tolerance.

DR. ALLEN: Thermal adaptive response. That is very active with measurements of aberrations and other kinds of damage.

DR. MacGREGOR: This is getting to--actually, I was going to make a different comment that relates to the same thing, I think, and that it would be useful in whatever is decided, in terms of the mechanistic study, to perform the same mechanistic measurement on the heat without irradiation experiments so that you can compare. That would help to dissociate those two things.

DR. TICE: It would be kind of funny if RF signals all gave a certain kind of micronuclei. Hyperthermia has already been reported to induce chromosome-type micronucleus rather than acentric fragments, so that would help a lot.

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DR. LOTZ: One of the things that we had on the list that that at least refers to is this whole idea of needing to do a more careful non-RF temperature profile in terms of the micronucleus response. There seemed to be pretty good agreement about that yesterday so I don't know that we need to talk about it a lot more. But it is on the list.

DR. OWEN: I guess the only thing we got so far this morning on that was maybe looking at a 3 to 4 degree C range and, perhaps, including also some temperatures below.

DR. ROTI ROTI: I would think you should go 37.5, 38, 39, 40, something like that.

DR. OWEN: At quarter degree increments or something.

DR. ROTI ROTI: I don't know about quarter. Half degrees.

DR. OWEN: Depending on what the exposure system can generate.

DR. ROTI ROTI: I would guess that you don't need to go real high because there is data at higher temperatures. It is the 38 degrees, 39 degrees. But, remember, all of our systems are talking about 37 plus-or-minus 5, so I think you actually want to do 37.5.

DR. WILLIAMS: And probably an arm of that study should include an RF exposure that you agree does not, by

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itself, in your systems, produce an effect. In other words, take a suboptimal RF exposure 1--

DR. ROTI ROTI: I am not sure I understand.

DR. WILLIAMS: In other words, you can do your heat experiments--one arm of that experiment is heat alone, but it should be heat, then, with a low RF exposure that you agree--

DR. ROTI ROTI: Right; I think we have to heat with RF and heat without RF.

DR. WILLIAMS: But one thing should be an RF exposure that you decide does not, in itself, in any of your experiments, produce--

DR. ROTI ROTI: I kind of think we have already done that.

DR. TICE: No; what Jerry is talking about is dressing the cells with heat.

DR. WILLIAMS: Shifting the curve.

DR. TICE: And then throwing on top a suboptimal RF exposure.

DR. OWEN: Perhaps shifting the dose-response curve.

DR. ROTI ROTI: Oh, yes; that kind of thing we can do.

DR. OWEN: With non-RF heat.

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DR. MOROS: You can use such a low SAR that you know you are not going to heat--

DR. ROTI ROTI: It is not so easy to do that, in a sense, because what I plan to do for the temperature response is a highly controlled system. It is very well established and very well characterized, but it is in a completely different lab than the RTL room. When we would heat with the RTLs, or heat in the RTL room, it is going to be thermally balanced in such a way that nothing else can be done in that room or else we will have to take an RTL and put it down in Eduardo's physics lab where it can be heated.

If you are going to be heating with an RTL, that means all of the experiments going on at 37 will have to be put on hold when you do that. These are just logistical. It is doable but it is--

DR. MOROS: It is same case for them.

DR. ROTI ROTI: Well, no; it is quite a bit different because they don't run concurrent studies. If we have ongoing transformation studies going on at the time we are trying to do this experiment, we are not going to be wanting to crank that room up to 39 degrees. Cranking that cold room up to 39 degrees is a big difference, because these are done in a hot room.

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So if we do this, we may have to construct a separate heating environment for a single RTL someplace in your lab.

DR. OWEN: One thing that we talked about a little bit yesterday and have not fleshed out any this morning but was one of the points from yesterday, and I would like to collect any additional input on, is doing experiments that take a finer look at exposure duration. Again, with the existing work, we have, in one case, 3-hour and 24-hour data. In the other case, we have, remind me, Joe, but it is something like 8, 24--

DR. ROTI ROTI: We have 3, 8 and 16 and 24.

DR. OWEN: 3, 8, 16 and 24.

DR. ROTI ROTI: I can tell you why we chose those is because the 24-hour is what they used. We were kind of following what the WTR did. I think 24 hours just became a natural choice. We chose, from the beginning of our study, to look at longer-term exposures of a day or more and, in general, quite often, 24 hours was our minimum, just going overnight.

Then, in the later stages of the study, we began to do more short-term studies. Our DNA damage data was all 2, 4, and 24 hours. And so we tried to at least get something on the order of the time intervals that we chose for the DNA damage studies.

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DR. LOTZ: What was the rationale in terms of the shorter ones, the 3 hours?

DR. ROTI ROTI: The 3-hour one, I think it just fell in the middle. But the 2-hour point that we picked was from the Lai and Singh experiment, basically, because they did 2 plus 4 hours.

DR. TICE: In vivo.

DR. ROTI ROTI: In vivo; right. And so we tried to do that in vitro.

DR. TICE: What drive our exposure duration side was the other experiments we were doing with the regular gene-tox battery where we were doing exposures for 3 hours and, at the same time--generally, in those particular batteries, you do 3 hours or 4 hours with and without metabolic activation.

Metabolic activation was never thought of as being necessary in this particular experimental paradigm but, at the same time, we didn't go to 4 because, since we are doing all the exposures in one day, if we did them in increments of 4, that meant that the day was running around 16 to 18 hours. By doing it for 3 hours, we could manage to do all the exposures we wanted to do within one day so, in a sense, that was our concurrent kind of situation.

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DR. OWEN: If I recall correctly, your protocol was to do the experiments at 3 and if there was an equivocal result, to do 24?

DR. TICE: Actually, we decided to do both regardless of what happened at 3. The reason for that was that if you look at the standard aberration protocol exposure, which is what we were doing on cycling cells, then that is generally a short exposure, and one that is considered a "1 and a half cell" cycles.

One and a half cell cycles in human lymphocytes is about 20 hours, or something like that. We were trying to mimic this one against the 20-hour one. The reason we went to go to 24 was, again, just because it was convenient. Sometimes, science is based on convenience as much as it is based on scientific rationale.

DR. OWEN: It looks like we are at a convenient minor lull.

DR. TICE: Was that because I was speaking?

DR. OWEN: No; I think not. Well, I won't speculate. But if we can take only ten minutes and be back here to resume our conversation, that will give people time to do what they really have to do.

[Break.]

DR. OWEN: We will resume this discussion. Amongst the topics that we touched in yesterday that I would

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like to clarify input on is in vivo experiments. It was mentioned yesterday that experiments that are being planned could, perhaps, be designed to include micronucleus assays at an endpoint, our epic bioassays, for instance, that are being planned.

Another idea is that there may be--I think Dr. Roti Roti mentioned that, wow, he just missed the boat. He could have done those on an experiment that he was just doing. So a similar idea is there may be in vivo studies going on right now or just starting to which micronucleus assay could be added as an endpoint in some way.

So I would like to take some input on that, please.

DR. WILLIAMS: The experiments in France?

DR. LAGROYE: We did some experiments using head-only exposure. We used 1, 2 and 4 W/kg. That was a 2-hour exposure. We only looked at the DNNMH using the comet assay. Actually, we found a 20 percent increase in the comet length with the 4 W/kg SAR only.

DR. WILLIAMS: In what cells?

DR. LAGROYE: No; it was in rats.

DR. WILLIAMS: In rats, but what cells? You extracted what; brain cells?

DR. LAGROYE: Yes; brain cells.

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DR. VERSCHAEVE: That was whole brain, not particulates.

DR. LAGROYE: The whole brain. We followed the same procedure we used for the Lai and Singh replication study.

DR. MOROS: The question I think is anybody planning some--

DR. OWEN: No; the question is is there a call for doing in vivo experiments, particularly keeping in mind that if FDA were to recommend such, such a recommendation might be taken up by people who are just beginning studies or who are already doing studies. But the larger question is should in vivo studies be done with this endpoint, period. If so, should a purpose design the in vivo experiment to look at micronucleus assay await other in vitro information, and so on.

DR. TICE: Let me ask the question this way. If it turns out that the micronuclei-induced in vitro are all caused by localized hyperthermia, I know from what C.K. said that currently people are exposed up to, was it 1.6 W--

DR. CHOU: Up to 1.6 W/kg.

DR. TICE: Up to a 1.6 W/kg peak. So then the question you might ask is whether or not, at that SAR, would you expect some kind of localized--the same kind of heating phenomenon that we are getting at 5 and 10 because we are

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not picking it up at--well, we haven't picked it up below 5 yet; let's put it that way.

DR. CHOU: In addition to that, 1.6 is the limit for the cellular phone, for the general public, so for the uncontrolled environment. But for people in the profession, occupational exposure, in a controlled environment, the SAR peak limit is 8 market.

DR. MOROS: For how long? 8 for 6 minutes, I think it is.

DR. CHOU: Yes; at least 6 minutes, above.

DR. LOTZ: No; it actually go above 8 for 6 minutes, or less than 6 minutes. Beyond 6 minutes, there is no time duration to it. That was the only comment I was going to make about Ray's point was that, in Luc's introduction yesterday, the occupational studies, where there had been a suggestion, people who are occupationally exposed to other sources, not specifically the cellular phone, might receive RF exposures of higher, particularly to the extremities of higher fields certainly than 1.6 and for short periods of time, at least, maybe on a repetitive basis or something like that.

I think those occupational studies raise the question. And, at least for us, there is that concern of workers who have other RF sources. So it wouldn't specifically relate to the question of a cell phone and

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micronuclei but it would relate to the more general question of RF exposure and micronuclei.

DR. TICE: Those two epistudies--Luc, you have had two positive epistudies?

DR. VERSCHAEVE: Yes; we have two positive, but dosimetry was not well done so there is no idea about what--

DR. TICE: So then the answer, based on that information, would be yes.

DR. VERSCHAEVE: Yes.

DR. TICE: I think it would be important to do an in vivo study.

DR. VERSCHAEVE: I think so; yes.

DR. TICE: And it would probably have to be some kind of repetitive exposure because I don't know, if you did a 2-hour exposure and then did the normal sampling--well, you could do a 2-hour exposure and take out lymphocytes from animals, stimulate them to divide, as long as there is a whole-body exposure, I expect; right--because, if it was head only, I don't know--I realize the blood circulates to the head but, maybe if it is a whole-body exposure, there might be a greater chance or likelihood of picking up something.

I would like to see some of the more long-term exposures that are planned or are being done would be a really great way to see, under those chronic exposure

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conditions, whether there is accumulation of events across time.

I would be looking, if it was mouse, at least I would do both blood, peripheral blood, like Jim's group had developed a long time ago and also lymphocytes, because that is the population that we did in vitro and that could be stimulated to divide.

If it was rat, well, I would still do peripheral blood but I would probably focus more on reticulocytes or PCE's and NCE's, except the Japanese are still talking about doing rat peripheral blood. I just haven't had much luck with that yet.

DR. MacGREGOR: I would make two comments. To me, the key overriding question is can this phenomenon occur in vivo. So it seems to me that you would like to have in vivo information. I think there are technical issues to think about in vivo because of the nature of the cells and their replication kinetics, that the lymphocytes, where the observation has been made--

DR. OWEN: I'm sorry; I didn't hear that last bit.

DR. MacGREGOR: The lymphocytes where, presumably, as a major part of this cell population in which the current observations are made, are a mixed population most of which are not dividing in vivo. So, now you have a technical problem of having to expose quiescent cells and then having

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a time lag to get them out and then choose some artificial in vitro culture situation.

So I think that whole issue warrants some discussion. I guess I would agree with what Ray just suggested that if in vivo experiments were to be done, I would like to pose the general question, could the phenomenon occur in vivo and probably look at two different kinds of cells, the erythrocyte model, which is a convenient model because the cells go all the way through their whole normal in vivo process, two micronuclei in vivo. So you don't have this technical problem of how to do the experiment and expose only part of the cell cycle and then do another part under an artificial condition.

And then, also, try to do, in some way, lymphocytes because it is the cell population that is probably being affected. So that would require you to be able to expose--I guess mice would be the logical model that would be a small species in which both assays could be done.

DR. TICE: I would almost be a little bit concerned about doing the erythroid cells because of the fact that the bone marrow is relatively far removed from the skin, wherever the exposure is going on.

DR. MacGREGOR: That becomes an engineering technical question, I guess, whether the irradiation can be-

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-I don't know the answer. Maybe an engineer could comment on whether--

DR. LOTZ: if we are talking about these frequencies, there is plenty of penetration in terms of the whole-body exposure, especially in a mouse. Almost the exposure would be more uniform in a mouse than it would be in a larger animal at, say, 850 MHz or even 1900.

MR. BASSEN: That depends on the exposure.

DR. LOTZ: Well, sure it does.

MR. BASSEN: If it is near field, if it is a dipole--

DR. LOTZ: That is why I said whole body. I am assuming we would use some more standardized system to--even whether it was kind of the circular wave guide, but certainly a horn or something like that in the far field, or the type of thing that is being used for the PIM 1 study. Those are going to provide exposures that have a lot of penetration.

There is going to be nonuniformity, but there will be, certainly, plenty of penetration to the other tissues.

DR. LAGROYE: Yes; there is a piece of work in Europe by the Kuster, Nils Kuster. They are working hard about the exposure system for rats and mice, too. So I think they should be able to help.

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MR. BASSEN: Is there any concern about constraint of the animals causing a stress that might cause some sort of response?

DR. ROTI ROTI: I have an answer to that question.

MR. BASSEN: Because these animals should be rotated--if they are allowed to roam, the SAR is going to vary quite a bit.

DR. ROTI ROTI: We have experience with that. We have used this radial irradiation system and the rats were accustomed to the restraint devices for I think a month before we started the exposures. Once they are accustomed to it, there is no detectable stress on the animals.

DR. MOROS: They actually go in voluntarily.

DR. ROTI ROTI: They go in voluntarily and they go to sleep.

MR. BASSEN: These are tubes, so they are aligned.

DR. ROTI ROTI: They are tubes.

MR. BASSEN: They don't turn around in those holders?

DR. ROTI ROTI: When they are really little, they do, yes. But you have a series of those holders as they grow. For two years, we had to have a series of holders.

MR. BASSEN: Do you push a plunger in to keep--

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DR. ROTI ROTI: There is a little thing that goes against their backside and allows their tail to come out through.

DR. MOROS: We went through these hoops and loops about how you best do this. The paper has been published. It is two papers. One talks about design dosimetry. Everything was taken into account, event the amount of excrement that they were going to produce within a 4-hour period to make sure that they didn't get wet with their own urine, to make sure they had plenty of fresh air coming in through their nose. Everything was taken into account.

But this system was designed to preferentially irradiate the head. If we are going to use something like that, then we need to change it for whole body.

DR. CHOU: We did the same thing at City of Hope on this head-only exposure. We had a pretty tight system compared to the Washington University. That one, you have some leeway and the animal can move their head. The one we had, even very tight; we have a plunger in the back and it only took about a few days and they got used to it.

We had an adaptation period for seven days. There was no problem. Every time, the animal just goes in and they take a nap. So it was no real struggle. After a few days, they go in themselves. We just put the plug in there and they just stay in there.

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DR. ALLEN: What is the limit of the exposure period when you have this restraint?

DR. CHOU: Two hours of exposure.

DR. ROTI ROTI: We did four hours.

DR. MOROS: A day for two years.

DR. TICE: Can I ask the question; if the orientation is actually not a problem when you go to a chronic exposure, given the fact that if they are in the wave guide where the animals can move around, they are still exposed for longer durations but the orientation will, in a sense, have some average across time.

DR. HOOK: I would point out, for the in vivo, if you are trying to do bone marrow, you could do localized exposures, too. You could expose just the femur. That would probably allow you to get to higher SARs.

DR. MacGREGOR: I would just add rats and mice have been mentioned, but I would say that the mouse model offers a significant advantage in that the frequency of micronucleated cells in peripheral blood reflects that in bone marrow whereas, in the rat, it does not and you have other technical problems because of splenic removal of the micronucleated cells.

The other comment I would make is that I don't know the maximum times of restraints that are used but, often, animals are restrained and can even be fed in

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restrainers and their metabolism and excretions studies are done and those kinds of things. So there are restraint systems that would even allow the animals to eat and drink that are available that could be looked into.

DR. CHOU: The system that we have exposing the mouse is being used in Australia to replicate the study for checking the mouse lymphoma study. We have the system exposing 40 animals at the same time, 15 exposure systems exposing 1200 animals there now. This is the second year going on. So that system works very well.

DR. TICE: C.K., you just said that that exposure is currently going on?

DR. CHOU: Yes; it is a two-year study in Australia.

DR. TICE: These are mice?

DR. CHOU: Mice.

DR. TICE: There you go, from the standpoint of all we need is--actually, all anybody needs, not necessarily we--is just peripheral blood smears from those animals, and you would have, at least under those exposure conditions with those--whatever SARs are being used, you would have an answer. That is probably--if there is a way to push for that, that would definitely give some indication of whether or not this is a biological phenomenon that occurs.

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DR. MacGREGOR: Those smears can be based on about 3 microl of blood so all you need is a tiny tail prick.

DR. TICE: And we would be willing to fly to Australia to collect the samples.

DR. OWEN: Actually, we could send Michael there.

DR. FENECH: The lab is probably not more than ten minutes away from where we are.

DR. TICE: All you need to do is to take a little piece of the tail off and then make smear out of that. That's all it is.

DR. FENECH: I am sure whoever is running that experiment can do it.

DR. TICE: I think, to me, that would have a really high priority right now.

DR. FENECH: I think it is Tim Kuchel who is in charge of that study, I understand.

DR. CHOU: That study has two populations of animals. One group of 600 animals is the PIN 1 mouse. It is exactly the same species as Michael Repacholi used. The other one is a wild-type regular mouse. They have different dose power levels, SAR levels.

DR. FENECH: While we are talking on the in vivo aspect, perhaps we should at least consider the tissue that is being sampled relevant to the problem and whether any actual human in vivo work could be done.

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I just want to point out that, say, in the mouse studies, it is, in theory, feasible to do a micronucleus assay with cultured fibroblasts collected, let's say, from around the brain and so on. So that is one option. Or looking at the micronuclei induced in the skin is another possibility.

The reason why that might be relevant, at least with the mobile phones, the phone is held close to the ear. Maybe in the human situation, you could, again, in theory, sample cells from the skin as a possibility or, let's say, this hasn't been done before but maybe scraping of cells from the epithelium inside the ear is another possibility.

If you stretch your imagination even more, and this probably would be a big flop, but, anyway, I might as well say it. This is just for consideration. One could actually also place target lymphocytes, let's say, in a small test tube or capsule place in the ear while exposure from a mobile phone is occurring and then do the test on those cells afterwards.

All of this is really simply to mention that there are--theoretically, it is possible to do micronucleus assays in vivo in humans directly. But it hasn't been done so the feasibility of that is not entirely clear.

DR. OWEN: There were a few things from yesterday that I definitely need some clarification on, moving on to a

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new topic. But we can come back if we need to. There was some mention yesterday of what is needed in terms of comparing multiple technologies or evaluating the role of modulation and carrier frequency.

If I could, I would like clarification of what was said yesterday on that.

DR. TICE: That was something we threw in because of Carl Blackman at EPA during one of the conversations, I don't know, it seems like a decade ago by probably last year, where one of the--he is somebody who specializes in looking at radiofrequency work within EPA. One of the things that he talked about was trying to separate out modulation from carrier wave.

That is about as much as I know about it and probably the people who are on the physical side can make a better comment. But at least that is where the origin is.

DR. OWEN: Anybody else want to add to that?

DR. CHOU: That was in the WTR study, we had the different modulations, FM, TDMA, CDMA. That is the purpose, to cover the different possibilities.

DR. HOOK: The idea here was to have just a straight CW source, no modulation; the idea being that, then, that if the response vanished, it would be attributable to the modulation rather than the carrier rate,

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and that that might be a way of getting at the question of whether it is a thermal effect or not.

DR. CHOU: Whether it is a thermal effect or not, I think your control temperature study will help explain that.

DR. LOTZ: Is there any thinking that there is importance to studying one technology in terms of wireless signals over another. For your work at ILS, you didn't see any difference between the different technologies that you have tested so far, anyway, as I recall, in this particular assay, anyway, in terms of micronuclei.

DR. HOOK: Not if you look at just the peak, or what is giving us our highest response. But we don't have enough information to talk about, really, dose response at all. But, certainly, differences in dose research between different technologies is unclear.

If you look at it, we have only got really one experiment where we compare 10 and 5 between two technologies, and we got a difference there. So we don't have data.

DR. LOTZ: That goes back to my question, then; is there reason to preferentially study one--not necessarily one specific, but certain modulations over others.

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DR. HOOK: I think, based on our data, you would have to argue that you would want to look, at least in the beginning, all of them that we did.

DR. LAGROYE: At the last BEMS meeting, one group from Italy showed that using the GMSK modulation for the GMS signal, they could get micronuclei after 15 minutes exposure. So it was in human lymphocytes. That was a pretty preliminary study, but maybe there is something to look at here.

DR. CHOU: I would go back to that assay numbers. I have to put some qualifications here. We are talking about 10 W, 1 W, 5 W, whatever. Really, what we are talking about is the every pixel. It is based on the FDT calculation and you measure at one point. The number we quote, 1.6 or 8 W, the FCC level, that is the average over 1 G of tissue.

If you go to ICNIRP, and most European countries have adopted that ICNIRP standard, that is an average over 10 G of tissue. So if you have this average, all the numbers will be different depending on what standard you are looking at.

So, in terms of ICNIRP, their peak SAR is 2 W/kg for the general population averaged over 10 G where occupation is 10 W/kg over 10 G.

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DR. OWEN: Of course, their sample size here is never approaching 10 G so we are forced to average over the sample size. Or Ray would be a lot more pale. I don't know, C.K., if you want and can expand upon in the discussion of what type of signals to use.

In another context, I have heard some discussion of a model signal designed to be representative, somehow, more representative of I will say reality in quotes. Do you want to add to that?

DR. CHOU: Actually, I think the best person to answer is Mays Swicord. He is aware of all these MMF-supported programs around the world, all different modulations. He will give you better insight on that in terms of which ones--let's wait until he comes back.

DR. OWEN: There was another point that we needed some clarification on. I would like to get input again on what cell system should be used for these studies, or does it matter. Clearly, we have got two different cell systems that have been used in the data that have been presented here and I would like, again, to get clarification or input on that topic.

DR. TICE: Russell, my first reaction is that you are talking about trying to independently verify something in another lab you have got, of course, two approaches. One of them is you use a different system, a different cell. If

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you get the same response, then you have got a really robust response that is independent of anything. It occurs. That gives you some information.

If you do a different cell in a different system and you get negative responses, then you don't know if it is the system or the cell. Then you end up going back to the same system and the same cell but then in a different lab. In this particular situation, what we are kind of relying on is the fact that Joe has 5 W/kg positive reproducible response in 10T1/2 cells when they are quiescent.

Therefore, we already think that it is independent. We would like to think that it is independent of cell and independent of technology so long as you are at the right SAR. So if you go that route first, and it stands up, then you have already answered your robustness question.

If it doesn't stand up, then you have got to worry about the second level.

DR. ROTI ROTI: We chose to use the C3H 10T1/2 cell system because we could follow up with some "so what" questions in terms of the data we were already collecting, namely neoplastic transformation data and other endpoints that we have been measuring in this cell system. So, to us, it made sense to see if this effect applied to that system.

DR. WILLIAMS: Did you do transformation studies?

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DR. ROTI ROTI: Yes; it is summarized on that list.

DR. WILLIAMS: And they are negative?

DR. ROTI ROTI: Yes--well, at 0.6 W.

DR. TICE: Yes. There you go.

DR. ROTI ROTI: They are not done at a higher SAR.

DR. HOOK: The one thing that we do seem to have, though, is evidence that, unless you are dealing with either quiescent or plateau-phase cells or non-cycling cells, that you might not see this effect. So, although I would like to see other cell types evaluated, it would have to be one in which you can at least set up a system that is similar to what we have which is noncycling or plateau-phase cells.

DR. TICE: Except that we were not sure if Joe's negative data for the cycling cells was--

DR. ROTI ROTI: It is something we need to find out.

DR. TICE: Yes; so that is kind of a question.

DR. FENECH: I just want to comment that when you are using the primary lymphocytes, every time you use a sample from a different individual, you are actually looking at the different cell because the inter-individual variation and sensitivity is different. So that has to be considered. It is a question, then, do you do the test with lymphocytes or blood samples from people who we know are sensitive to

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another carcinogen--for example, X-rays, something like that. It is very easy to find out who is and who isn't sensitive and that reflects the DNA-repair capacity.

So it may be more instructive--first of all, you are working with human cells and you are looking at human cells of different sensitivities, both primary. I think that might be considered to be more relevant in some ways and more focused.

DR. ROTI ROTI: We have batteries of double-strand break repair-deficient cell lines in our laboratory and we can do these kinds of studies. Should it be deemed relevant, it is no problem for us to do these cells in matched mutants of wild type and specific repair-defective, double-strand break repair-defective cells.

DR. ALLEN: Joe, is it known whether your 10T1/2s are P53 mutant or--

DR. ROTI ROTI: I think they are not.

DR. ALLEN: You think they are one of those that are normal P53?

DR. ROTI ROTI: Yes; I think it is normal P53. That is another thing that can be done in P53-positive and P53-negative.

DR. ALLEN: Right. There is a building database on the differences in response.

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DR. ROTI ROTI: We have those available, but I don't know if they are available in the C3H 10T1/2 cells. Those were actually discussions we have had of doing the transformation studies in different genetic knockouts of cancer suppressor genes. You keep talking about doing studies. You could do them forever.

I think it makes sense to do it, but it a little bit depends on the results of the PIM mouse replication. I think those studies might be very relevant should that result be replicated.

DR. CHOU: We did the P53 study at City of Hope using the same system and the result was negative. We were using the Petri dish method.

DR. HOOK: Do you remember what was the cell line? Just looking for P53 induction.

DR. ROTI ROTI: We are talking about in a P53 knockout cell line where you have a mutant P53 and then you actually try to see if the field affects the transformation frequency when you suppress certain oncogenes. We are kind of getting off the subject here, but it might be relevant if you find that those things affect micronuclei formation and you find that, in the PIM mouse, it is sensitive to the field whereas the wild-type mouse isn't.

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Then those kinds of studies become relevant in vitro because then you can begin to map what defects would interact with fields and what defects wouldn't.

DR. OWEN: The only other thing that we have not touched on at all in terms of clarifying something that went on earlier and this is not, again, necessarily an overarching topic but a point, and that is the question of whether consider of micronucleus-formation ratios or the number of cells affected is important to require or to specify in the conduct of a study.

Presumably, one could call for both.

DR. TICE: We actually collect data on both. But the normal analysis that is done is the frequency of cells with micronuclei because it is binomial. When you start talking about numbers of cells with different frequencies of micronuclei, you can look at the dispersion among cells to see if it is--because, if it is overdispersed, that gives you different kinds of information.

But, generally, both data would be collected because both might be informative.

DR. ROTI ROTI: We collect both.

DR. FENECH: Collect all the data you can.

DR. OWEN: I am going to back through just the general topics at this point that we have covered this morning, roughly in reverse chronological order.

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We talked about which cell systems should be used. We talked about considerations of which signal or technology, or which signals, should be used for more research. We talked extensively about the type of in vivo research that is warranted.

We talked quite a bit--well, we talked a little bit--about non-RF heating and cooling experiments. We talked about exposure duration. We talked about experiments to look at the mechanism is micronucleus formation that is responsible for the observed RF and non-RF heating effects.

We talked about exposure-system considerations and further characterization that might be warranted. We talked about experiments using ambient temperature offset. We talked about dose-response evaluations and we talked about the overarching concept of repeating work that has been done with multiple labs, multiple exposure systems, more than one lab, more than one exposure system. Related to that was additional analysis of available data.

At this point, I would like to ask the group if there are any overarching topics that you recall from yesterday that I have missed in that list or that we haven't yet touched upon that relate directly to follow-up work on in vitro micronucleus-formation experiments that we saw the results of yesterday.

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DR. MacGREGOR: I can't think of overarching questions but there are few small points that were mentioned yesterday in the context of some of the things we talked about today that we didn't mention yesterday, so maybe I will just mention those. One was that among the mechanistic questions raised yesterday was--I think it was on Ray Tice's list whether free radicals were involved and there was, apparently, reason to think they might be. I am not sure I quite caught what the reason was. That was one thing mentioned.

Another thing mentioned yesterday was in the context of using the heat-shock response that, perhaps, that could be done a little more globally by using microarrays to really look at stress pathway response and characterize that, so I guess that issue was, if you are going to do those kinds of experiments, does it or doesn't it make sense to try to expand your pathway mapping or not.

Then the third thing I can think of is brought up by Mike Fenech was the idea of low folate. I guess there are two ways you could think of low folate. One is just individual sensitivity. Another might be signal amplification because, for many kinds of agents, if you lower the folate in the medium, you may raise the background a little bit off and you raise the response even a lot more than the background.

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So it could be to think about varying that parameter could conceivably both amplify the signal and your dose response and, also, could be relevant to individual variability issues. I guess those are three things that were put on the table yesterday that we haven't talked about yet.

DR. OWEN: Any others?

DR. ROTI ROTI: I would just like to say one thing about the list. I think one needs to be aware that HSF activation, to my knowledge, is the only parameter related to gene expression, and so on, in which the thermal response has been pushed--I don't think we have pushed it to its limit, but it is pushed to a limit that shows that it is relevant at the kinds of temperatures that we might expect in a thermal artifact situation.

Other kinds of gene expression and microarray technology would all have to be characterized as part of the research in that regard. So the thermal standardization of those assays would need to be done.

DR. MacGREGOR: Let me point out, I wasn't advocating any of those three. I was just getting them back on the table.

DR. ROTI ROTI: I would love to have funding to do microarray studies in RF fields and low temperature. So if you guys put that on the table, wonderful.

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DR. ALLEN: In the same context with the arrays and the folate is the age because microarrays are showing age-dependent levels of expression. So I think if we are going to suggest getting into that in a big way, that really needs to be taken into account.

There are changes, for example, in cell-cycle regulation and stress response and repair systems. It is all very important here.

DR. MacGREGOR: I guess I would maybe, since Joe raised the question, offer a personal opinion that all three of those, in my mind, might be more secondary kinds of issues that, if you get into mechanistic studies, it would nice to know but maybe wouldn't be the first kind of thing you want to think about.

DR. ROTI ROTI: I really think the reason I put the HSF on the table is it is potentially a good indicator of temperature rise in the system. We have spent a large number of minutes discussing where the temperature rises are and are they significant at any level.

DR. OWEN: And the biological thermometer was explicitly mentioned this morning in the context of doing those temperature characterization experiments.

DR. ROTI ROTI: Right. I think that is an important--I mean, I would like to distinguish the use of that as an indicator of thermal artifact versus

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investigating the genetic changes that might be induced by RF fields. Those are two different studies.

DR. MacGREGOR: I would certainly agree with that and I would agree that it is important to characterize the heat-shock response as a function of the nonirradiation treatments to see how good an internal dosimetry it is to heat and then, if that proves out, then incorporating that into all the studies would be extremely valuable.

DR. ROTI ROTI: That is what I think.

DR. FENECH: If I understood correctly from what you were saying yesterday, I thought you were suggesting that the heat-shock protein or the RNA ideally would be also measured in the binucleated cells in your scoring the micronuclei.

DR. ROTI ROTI: That is probably not feasible as I thought about it longer. What is feasible is to look for HSF activation as a measure of heating in the system. That is number one. But that doesn't give you any information about where in the system the cells are.

In our system, where the cells are attached, it might be possible, if the expression of a reporter molecule that is fluorescent is as sensitive as the activation of the transcription factor, then we could map the hot spots and also we could sort the cells prior to adding cytochalasin because they would have to be sorted immediately when they

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come out of RTL to see the elevated levels of the heat-shock proteins.

Then we could actually sort the ones that were not heated.

DR. FENECH: They are sorting them with flow cytometry?

DR. ROTI ROTI: Yes.

DR. FENECH: That treatment, itself, might cause--

DR. ROTI ROTI: That is why you do shams, because we found that, unlike RF where we couldn't detect any DNA damage, if we stained the cells with Hoechst and sorted them with a flow cytometer, we can measure comet, DNA damage by the comet assay.

DR. HOOK: What temperature resolution do you think you can get using the heat shock?

DR. ROTI ROTI: You can measure 5 percent of the cells heated at a degree. We probably might be able to try a half degree just to see if we can push it farther. I mean, a half degree would be more important than a degree, in a way. But that is only in a small fraction of the population. That is 5 percent of the cells were heated. The rest weren't.

DR. HOOK: Do you only one type of cells and look at them?

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DR. ROTI ROTI: Yes--well, no, you dilute. You dilute with unheated sample.

DR. OWEN: That was done by diluting the extracts.

DR. ROTI ROTI: We need to do it by diluting the cells.

DR. OWEN: At this point, I, just a few minutes ago, went over the main topics that we have talked about. In the remaining 10 minutes, 15 minutes, I guess--well, maybe the next 5 or 10 minutes--I would like to hear some input about the relative priority of the different types of studies that we have discussed, these different topic areas.

Don't all talk at once.

DR. WILLIAMS: It seems to me the first priority is is it a real effect. They have repeated data but the number of repetitions is not high. So the first thing that needs to be done, I think, is to determine is it an effect and the second thing is is it heat or is it the irradiation, or is it a combination of the two.

If it is heat, then there are a whole series of studies that you then go forward with, studying heat but, at the same time, studying the ability of the different technologies to induce patterns of heat in an individual. If there is a irradiation effect, then it requires stepping back, coming up with a hypothesis as to what the interaction could be and what would be the mechanism of it by which the

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irradiation couples to the cells and, in some way, detect it has been irradiated. That is a more complex and varied approach, I think.

DR. ELDER: I would suggest that the second priority would not be the mechanisms but the second priority would be to an in vitro study because I think we are looking at an applied research program here. We need to know whether or not emissions from cell phones are causing an adverse health effect. We don't need to wait until people do a lot of mechanism-type studies before we try to get an answer for the because we all know that arsenic causes cancer by, to my knowledge, we don't have too many clues as to how arsenic causes cancer.

DR. OWEN: I'm sorry; did you say an in vivo study?

DR. ELDER: In vivo; yes. That would be important, I think, for the second-level priority, too. And the mechanisms would fall after that.

DR. HOOK: I think repeatability, but what I mean by that is not us doing four tests at 10 but someone else doing tests to demonstrate robustness. It becomes, what, best out of 7, or something, if we just keep repeating this. I don't know if it would get stronger if we do it three or four times. If someone else does it, that gives us a lot of strength.

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I think even before you go to in vivo, we want to look at a dose-response curve. That will tell us. And if there are differences between the technologies that would lead us to want to do more than one technology in the in vivo study. We want to have as narrow a focus on the in vivo study as you can. These are very expensive if you go to the in vivo case.

We would know what we would need to test in vivo, and then that would be next.

DR. ELDER: You would expect some guidance on the in vivo studies from the replication dose-response studies that you would do at phase 1.

DR. HOOK: Right. So I am saying, repeat, dose response, and then go in vivo.

DR. MacGREGOR: I would point out that if there are ongoing studies, then sampling and measuring the micronuclei is a lot cheaper than any of your in vitro studies and ought to be extremely high priority.

DR. CHOU: To go back to point 1 on the heating study, I want to keep emphasizing we should also include the cooling study, too.

DR. WILLIAMS: I would be less enthusiastic about moving to in vivo studies until we know, really, what we are testing. Normally, if you set up an in vivo, you go to 5, 10, 100 times expected human exposure. We can't do that.

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If we could do this in a straightforward toxicological way, that would be the preferred way. We can interpret the animal studies. We have huge databases.

But we can't do those experiments in the normal way. We can't multiply up the exposure levels. The confounder there is heat. I think it will be difficult to proceed to the in vivo studies without understanding whether we are really looking at a heat phenomenon or an RF phenomenon or an interaction between the two.

DR. OWEN: Two subfactors of that become--one, I think we are trying to draw some distinction between design of new in vivo studies to look at micronucleus and looking for good opportunities to add on to existing studies at low cost. At least that is something to consider.

DR. MacGREGOR: I guess I would restate what I think I said. I think I would agree with what Jerry said about initiating new studies in vivo. That is complicated and you need to understand what is going on in vitro to design that. But I think it should be very high priority to identify ongoing in vivo studies and obtain relevant samples from those knowing what we know at this point.

DR. CHOU: I wish Mays Swicord was here because now there is a big problem going on in Europe called Performer A. It is directly related to these long-term in vivo studies. This can be integrated into that.

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DR. OWEN: I think I have his phone number. Thanks. I will talk to him one way or other.

Another thing to note, just in terms of keeping in context, adding on to studies that are already designed, we would want to take into consideration that, since we are not defining the design of the studies, if they were negative, they may no be very informative, may not be informative hardly at all. But certainly the high priority is tied with the possibility of detecting something if it is--

DR. ROTI ROTI: Again, the caveat is that even the studies that are going on now are not at the SAR levels that they use to see the effect. So, even if you tie it into existing studies, it may not tell you anything because we know, from in vitro studies, you need to get to about 5 W/kg.

But if you go to 5 W/kg, then you are going to have to get an animal that can handle it.

DR. LOTZ: The one caveat on that, Eduardo, I would put are, again, these--and they are only preliminary--but they are these human studies with, obviously, a small number of people. But it is not likely that they are getting any kind of steady exposure to 5 W/kg.

So they provide a hint that I think might tie into the rationale to add on to existing in vivo studies even though their SARs may be low.

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DR. MOROS: Oh; I agree you should collect the data, but we aware of the--

DR. HOOK: In terms of new in vivo, first we have to know what the dose response is. Maybe we don't need to go to 5 or 10. Second, we know that, at least for head exposures, if we focus it on the head, we can go up to 10 W/kg in the head. So we can do high localized exposures.

DR. LOTZ: I think we have covered this in some ways but, Graham, that point brings me back to another question. Is there a reason in vitro to go longer than 24 hours? Is there any point in doing that?

DR. HOOK: The other side of the dose response.

DR. ROTI ROTI: We can do that. That is maybe where the RTL has an advantage and perhaps we should do some studies of multiple-day exposures because we can't push the SAR up right away. But we can certainly keep the temperature well-controlled for multiple days. And maybe we would then get more comparable data. We could easily do that, especially in plateau-phase cells.

DR. MacGREGOR: I wonder if any mechanistic discussion around this point would be valuable. We don't understand the mechanism but we do understand that micronucleated cells get eliminated from the population with a certain kinetics and generally come to steady state after a couple of cell cycles.

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So I would say probably, just off the top of my head, probably you would want to think about doing experiments that span maybe a few cell cycles and longer than that may not be that beneficial.

DR. ROTI ROTI: I was thinking of doing this in plateau-phase cells.

DR. MacGREGOR: Well, okay.

DR. ROTI ROTI: I wouldn't want to do that in exponentially growing cells. First of all, we didn't find an effect there but we did find it in plateau-phase cells. There are a couple of other reasons for trying to do some longer studies in the plateau-phase cells. First of all, we do a 6-week exposure to a plateau-phase C3H 10T1/2 cell when we are doing to transformation assay anyway.

So it would make sense to do that in a plateau-phase C3H system. It wouldn't make sense in an exponentially growing cell.

DR. MacGREGOR: I agree.

DR. WILLIAMS: This is just a general comment and it isn't meant to be negative at all, but I do think that you can get in trouble planning experiments when you don't have a hypothesis. Whatever the mechanism, if there is a mechanism working here, we know it is not like ionizing irradiation. We have a lot of negative data.

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Whatever we come up with our overall research plan, it has to take cognizance of that negative data. It does not produce chromosome aberrations. It does not produce mutation. If we agree that those data are real, we really have to come up, then, with a mechanism. Otherwise, we are fishing. That sometimes really leads you down the wrong pathway.

DR. CHOU: If we worry about if this can lead to possible cancer induction, there are many studies in the past. The Air Force supported many studies in the '80's. Like you are saying, what are we looking for.

DR. WILLIAMS: In the long term, and, again, from a toxological perspective, is seems our goal finally is to say whether the use of cellular phones, as we foresee them in the future, produces a risk to an individual greater than the other risks that they experience in their lives. That is the general philosophy behind protection.

General risk is 10^{-4}, 10^{-5} that, when I am driving home to Baltimore today, I will get run over or something. So, when you are planning, especially your animal studies, and you have to say can we pick up a risk of this level, like with EMF, at some point, we have to say our toxological system simply does not pick up a small risk that is unlike the other risks we have assayed in the past.

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If it is some unusual mechanism, we have no experience at appreciating that in toxicology. So, again, I would just--we have all thought about this, but I think we all have to think again; what is the real hypothesis that we are testing. What are we really looking for and what is the most direct way to reject or defend that hypothesis.

DR. OWEN: Given that we have very little time left, I would just like to solicit one more piece of input and that is, in the diagram of relative priority that I was sketching out on this piece of paper, I did not have a specific mention from anybody about a couple of topics that we had before, that being additional characterization of the existing exposure characteristics and exposure-system modification or development.

DR. WILLIAMS: It depends on your hypothesis and what you want to test.

DR. LOTZ: I guess this sort of relates to what Joe said a few minutes ago, too. I think we have got a couple of not perfect but pretty good exposure systems here. It is important to press ahead with the biological experiments. If we can, in parallel, improve exposure systems, that is good but I don't think we want to wait. In a sense, we did that some years ago.

DR. ROTI ROTI: We need to do both.

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DR. WILLIAMS: Eduardo, are there specific benefits you see to improving any of these systems other than more specificity and more accuracy?

DR. MOROS: The benefit of the studies that I have envisioned are to allow the RTL to go to very high SARs over the UHF spectrum. In doing so, you also create a lot of SAR dosimetry data that one can then publish and then would be in the literature as a reference to what is used, SAR distributions at multiple frequencies.

So that is that. But this is sort of not a biological project but more of an engineering physics project. I do have plans for that. In fact, I have already an application submitted to NIH which will be reviewed during the next cycle. I don't know in whose hands it is going to fall.

I don't know if that is the best review for that type of application in view of this initiative. It may be more appropriate here. I don't know. We'll see. Whatever we do, though, I think that Dr. Chou said yesterday, and I agree completely with him, we need to characterize our systems redundantly. We need SAR. We need temperature. We need measurements. We need simulations.

We need all these things so that we don't have to go and get the data after the biological endpoint has been found, but the data is there. You just have to look at it

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and decide. Then, that will really erase from the minds of critics whether there was a thermal effect or not. That is what I am trying to provide to my biological thing is to make sure that we don't have a thermal impact on the cells.

DR. ROTI ROTI: I need to say something. I think the very first statement that was put on the table for a priority this morning was cross replication of the findings. I am not comfortable with our attempt to replicate the WTR data without being able to irradiate in the RTL system which has got a completely different geometry, a completely different set of whatever artifacts are there without being able to go 10 W/kg and compare the results.

I really think that upgrading the RTL irradiation facility so that it--and C.K. first asked me, why don't you just crank up the amplifier, buy a bigger amplifier. The reason is the absorber foam will probably melt. So we have got to figure out a way to get the thing more efficient so that we don't melt the absorber foam but still can get the right W/kg.

I think that if these effects are artifacts, maybe that is not so critical. But if these effects are verified, then having the irradiator system that can perform at these different SARs would be a very important tool for ongoing research, to follow up whatever these observations turn out to be.

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DR. WILLIAMS: You could do 5. You could take their cell system--

DR. ROTI ROTI: We have done 5. We can do 5. We can do more work at 5. We can do that right now.

DR. WILLIAMS: Is there any reason why you could not do the 10T1/2 cell?

DR. ROTI ROTI: Pardon?

DR. WILLIAMS: I was asking Ray whether there was any reason why they cannot do the 10T1/2 cell.

DR. ROTI ROTI: Yes; they can do them.

DR. OWEN: They would have to remodify the exposure system.

DR. TICE: But remember the cells have to be quiescent.

DR. ROTI ROTI: They can be trypsinized.

DR. TICE: But they have to be quiescent during the exposure--

DR. HOOK: Would we have to do it in plate, but we can do plate.

MR. BASSEN: There is a high-power absorber that you can use to substitute very easily.

DR. ROTI ROTI: That is a possibility.

DR. MOROS: We have the highest-power absorber that there is to be found in the United States, at least five years ago when we constructed the RTLs. Whatever we

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do, it means a not-trivial engineering task. That is what I am trying to say. Whatever we do to be able to go up to higher SARs is not going to be a trivial thing.

I have got at least 100 ideas that I can list, things I can do to get rid of the heat and to increase SAR. But I cannot do them in a week's time. So that is my point. It is going to have to be part of our project. Maybe some biological experiment can start while we work on the RTLs so that six months or a year later, then they can start working on a higher SAR levels.

MR. BASSEN: You can expose to 10 W/kg, can't you? No? I would think that is important.

DR. ROTI ROTI: That is important.

DR. MOROS: That is what we have been talking about.

MR. BASSEN: But you said with the exchange in absorber--I think it is worth the engineering effort, then, to get up to that level.

DR. ROTI ROTI: Yes; that is what we are saying.

DR. MOROS: That is what we are saying.

DR. ROTI ROTI: We can do 5 and have done 5 and can do a lot of perturbations with the 5, and we can probably do very quickly some strategies to expand the availability of positions that are irradiated at 5 W. But

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to get it all up to--I think 10 is what Eduardo would need some time to do.

MR. BASSEN: Yes; but I would encourage you to factor that into the proposal.

DR. ROTI ROTI: That is what we would do; we would factor it into the proposal, but I think that is part of priority because I think that would apply across the board.

DR. CHOU: The other system is much easier. I don't know--can you guys use the Petri dish? Then it would be a much more uniform SAR and the cooling is much easier because it is right at the bottom and the air flows right through it.

DR. HOOK: At least, the lymphocyte, we have a generator strong enough to bring up back up to 10.

DR. OWEN: I think we have probably gotten enough on that subtopic for the moment and we have reached the end of our available time for this meeting.

I want to thank all the participants, all the members of the Working Group, for coming and for your continued work on this topic with us. I want to thank the presenters for their time and thoroughness. I will remind them that I would like to have copies of the manuscripts that have not been published, if possible.

I would like to thank very much the people that organized this meeting, in particular Abiy Desta who carried

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really the bulk of the organizational load for us, and also the people here in this building in the conference organization office.

Thank you all for your attention.

[Whereupon, at 11:35 a.m., the meeting was adjourned.]

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